JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Radical Scavenging and Singlet Oxygen Quenching Activity of Marine Carotenoid Fucoxanthin and Its Metabolites

NAKKARIKE M. SACHINDRA,[†,‡] EMIKO SATO,[§] HAYATO MAEDA,[†] MASASHI HOSOKAWA,[†] YOSHIMI NIWANO,[§] MASAHIRO KOHNO,[§] AND KAZUO MIYASHITA*,[†]

Faculty of Fisheries, Hokkaido University, 3-1-1 Minato, Hakodate 041-8611, Japan, New Industry Creation Hatchery Center, Tohoku University, 6-6-10 Aoba, Aramaki, Aoba-ku, Sendai 980-8579, Japan, and Central Food Technological Research Institute, Mysore, India 570020

Antioxidant activity of carotenoids is suggested to be one of the factors for their disease preventing effects. Marine carotenoids fucoxanthin and its two metabolites, fucoxanthinol and halocynthiaxanthin, have been shown to exhibit several biological effects. The antioxidant activities of these three carotenoids were assessed in vitro with respect to radical scavenging and singlet oxygen quenching abilities. The 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity of fucoxanthin and fucoxanthinol was higher than that of halocynthiaxanthin, with the effective concentration for 50% scavenging (EC_{50}) being 164.60, 153.78, and 826.39 μM, respectively. 2,2′-Azinobis-3-ethylbenzo thizoline-6-sulphonate radical scavenging activity of fucoxanthinol (EC_{50}, 2.49 μM) was stronger than that of fucoxanthin (EC_{50}, 8.94 μM). Hydroxyl radical scavenging activity as measured by the chemiluminescence technique showed that the scavenging activity of fucoxanthin was 7.9 times higher than that by fucoxanthinol, 16.3 times higher than that by halocynthiaxanthin, and 13.5 times higher than that by α-tocopherol. A similar trend was observed when the hydroxyl radical scavenging was assessed by the electron spin resonance (ESR) technique. ESR analysis of the superoxide radical scavenging activity also showed the superiority of fucoxanthin over the other two carotenoids tested. Singlet oxygen quenching ability of the three carotenoids was lower than that of β-carotene, with quenching rate constants (k_Q, $\times 10^{10}$ M^{-1} s^{-1}) being 1.19, 1.81, 0.80, and 12.78 for fucoxanthin, fucoxanthinol, halocynthiaxanthin, and β-carotene, respectively. The higher radical scavenging activity of fucoxanthin and fucoxanthinol compared with halocynthiaxanthin is assumed to be due to presence of the allenic bond.

KEYWORDS: Fucoxanthin; fucoxanthinol; halocynthiaxanthin; DPPH; ABTS; superoxide; hydroxyl radical; singlet oxygen; ESR

INTRODUCTION

One of the important characteristics of carotenoids is their ability to act as antioxidants, thus protecting cells and tissues from damaging effects of free radicals and singlet oxygen. The free radicals and singlet oxygen produced in the body by the normal aerobic metabolism are highly reactive (*1*). These oxidants can react with various components of living cells, such as proteins, DNA, or lipids, and cause structural changes leading to many diseases; carotenoids have been found to be important in protecting against diseases and age related phenomena caused by oxidants (*2*, *3*). Antioxidative properties of many of the carotenoids, particularly that of β-carotene, have been established (*4*). The antioxidant mechanism of carotenoids is attributed to their ability to quench singlet oxygen and scavenge free radicals (*5*, *6*).

Fucoxanthin (**Figure 1a**), a major marine carotenoid, occurs in edible brown seaweeds. Fucoxanthin is metabolized to other carotenoids in marine organisms (*7*). In sea squirt *Halocynthia roretzi*, fucoxanthin is metabolized into fucoxanthinol (**Figure 1b**) and halocynthiaxanthin (**Figure 1c**) (*8*). Sugawara et al. (*9*) also demonstrated that fucoxanthin is hydrolyzed to fucoxanthinol during absorption by Caco-2 human and mouse intestine cells. These marine carotenoids are known to have beneficial biological effects. Fucoxanthin has been shown to have anticancer (*10*–*12*), antihypertensive (*13*), anti-inflammatory (*14*), and anti-obesity effects (*15*, *16*). Both fucoxanthinol and halocynthiaxanthin, isolated from the sea squirt *H. roretzi*,

* Corresponding Author. E-mail: kmiya@fish.hokudai.ac.jp.
† Hokkaido University.
‡ Central Food Technological Research Institute.
§ Tohoku University.

10.1021/jf071848a CCC: $37.00
Published on Web 09/26/2007

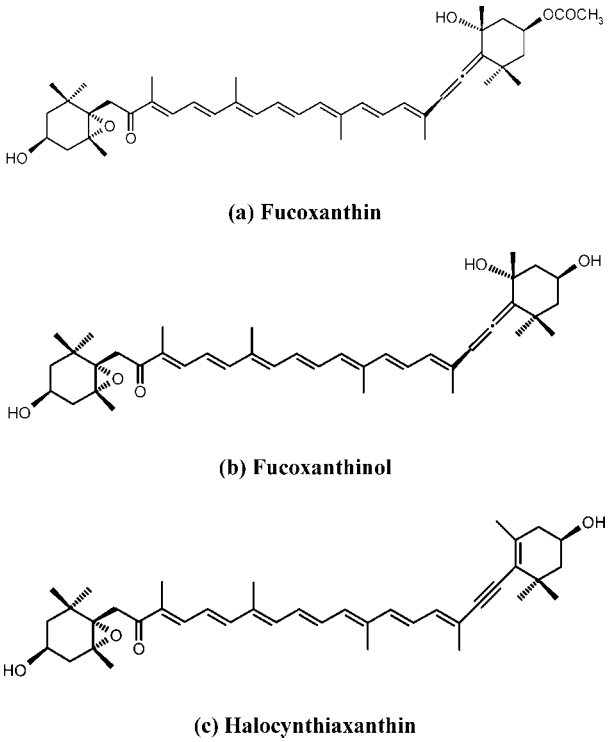

Figure 1. Structure of carotenoids.

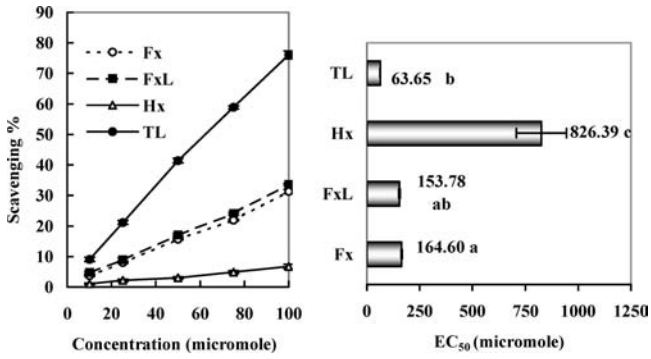

Figure 2. DPPH radical scavenging activity of carotenoids and α-tocopherol. Fucoxanthin (Fx); fucoxanthinol (FxL); halocynthiaxanthin (Hx); α-tocopherol (TL; mean ± SD; $n = 4$). Bars with different superscripts differ significantly ($p < 0.05$).

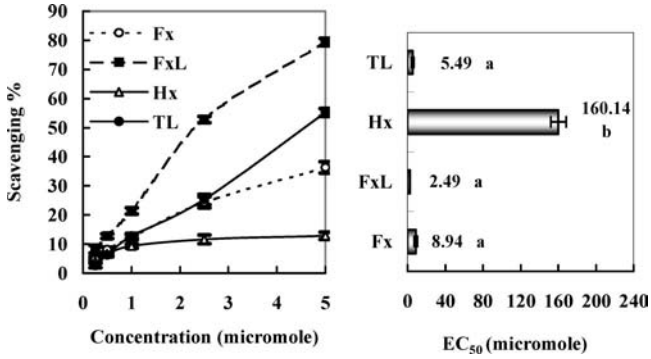

Figure 3. ABTS radical scavenging activity of carotenoids and α-tocopherol. Fucoxanthin (Fx); fucoxanthinol (FxL); halocynthiaxanthin (Hx); α-tocopherol (TL; mean ± SD; $n = 4$). Bars with different superscripts differ significantly ($p < 0.05$).

were found to inhibit the growth of HL-60 human leukemia cells, MCF-7 human breast cancer cells, and Caco-2 human colon cancer cells (*17*).

With respect to the antioxidant activity of fucoxanthin, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of fucoxanthin isolated from the diatom *Phaeodactylum tricornutum* has been demonstrated (*18*). The quenching ability of fucoxanthin from the brown seaweed *Hijkia fusiformis* against the organic radicals DPPH, 12-doxyl-stearic acid (12DS), and the radical adduct of nitrobenzene with linoleic acid (NB-L) has been studied using the electron spin resonance (ESR) method (*19*). The study indicated that, in the presence of fucoxanthin, the ESR signals for the DPPH, NB-L, and 12-DS radicals were reduced by 28, 57, and 66%, respectively. Yan et al. (*20*) demonstrated the strong DPPH radical scavenging activity of organic extracts from different edible seaweeds and reported that fucoxanthin was the active compound.

As many of the biological effects of carotenoids are related to their ability to scavenge reactive oxygen species, in the present study we investigated the scavenging activity of fucoxanthin, fucoxanthinol, and halocynthiaxanthin against different radicals and also their ability to quench singlet oxygen.

MATERIALS AND METHODS

Chemicals. DPPH, 2,2′-azinobis-3-ethylbenzo thizoline-6-sulphonate (ABTS), hypoxanthin (HPX), superoxide dismutase (SOD), allopurinol, 4-hydroxy-2,2,6,6,-tetramethyl-piperidine 1-oxyl (TEMPOL), 1,3-diphenyl-isobenzofuran (DPIBF), and luminol were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Xanthine oxidase (XOD from cow milk) and 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) were purchased from Labotec Co., Ltd. (Tokyo, Japan). All the other reagents used were of analytical grade.

Carotenoid Preparation. Fucoxanthin was isolated from the brown algae, *Undaria pinnatifida*, as previously described (*12*). Fucoxanthinol was prepared from fucoxanthin by hydrolysis with lipase (*16*). Halocynthiaxanthin was isolated from the sea squirt, *H. roretzi*, by a published method (*17*). The purity of the isolated carotenoids was checked by HPLC analysis.

DPPH Radical Scavenging Activity. DPPH radical scavenging activity was measured by the method of Duan et al. (*21*). A 2.0 mL portion of the sample in methanol was mixed with 2.0 mL of 0.16 mM DPPH in methanol and incubated at 37 °C for 30 min in the dark. After incubation, the absorbance was measured at 517 nm, and the scavenging activity was calculated as follows:

$$\text{Scavenging}\,(\%) = [1 - (A_{\text{sample}} - A_{\text{sample blank}})/A_{\text{control}}] \times 100$$

ABTS Radical Scavenging Activity. ABTS radical solution was prepared by mixing 5 mL of ready-to-use ABTS solution with 100 mL of acetate buffer (0.05 M, pH 4.5) and 5 units of peroxidase and incubating at 37 °C for 15 h. The decolorization of the ABTS radical solution was started by mixing 1.9 mL of ABTS solution with 0.1 mL of the sample and incubating it at 37 °C for 1 h. The absorbance was measured at 734 nm initially and at the end of the incubation period. Scavenging activity was calculated as follows:

$$\text{Scavenging}\,(\%) = [1 - (A_{\text{sample}} - A_{\text{sample blank}})/A_{\text{control}}] \times 100$$

Hydroxyl Radical Scavenging Activity. Hydroxyl radical was generated by the Fenton system, and the scavenging activity was measured by the luminometric method (*22*) using an ATTO Luminescencer 2300R (ATTO Corporation, Japan). The reaction mixture contained H_2O_2 (2 mM), $FeSO_4$ (0.1 mM), luminol (0.8 mM), and the sample in acetonitrile in a total volume of 100 μL. Acetonitrile was used instead of the samples in control mixtures. The 96-microwell plates with all the reagents except H_2O_2 were incubated at 37 °C for 5 min in the instrument, and the reaction was started by the automatic addition of H_2O_2. The resulting luminescence was measured every minute for a period of 30 min. The total luminescence count for the entire period was used to calculate the scavenging percent as follows:

$$\text{Scavenging (\%)} = 100 - \frac{(100 \times \text{Luminescence counts of sample})}{\text{Luminescence count of control}}$$

ESR Analyses for Hydroxyl Radical Scavenging. Hydroxyl radical scavenging activity of carotenoids was also evaluated by ESR analysis of the DMPO-OH (a spin adduct of DMPO and hydroxyl radical) in the presence and absence of the carotenoids by the method described by Niwano et al. (*23*). The reaction mixture contained 50 μL of 2 mM hydrogen peroxide dissolved in 0.1 M phosphate buffer (PB), 50 μL of 89 mM DMPO dissolved in pure water, 50 μL of each carotenoid in acetonitrile or solvent alone, and 50 μL of 0.2 mM $FeSO_4$ dissolved in pure water. The reaction mixture was mixed in a test tube and transferred to the ESR spectrometry cell, and the DMPO-OH spin adduct was quantified 113 s after the addition of $FeSO_4$. Signal intensities were evaluated from the peak height of the second signal of the DMPO-OH spin adduct. Measurement conditions of ESR (JES-FA-100, JEOL, Tokyo, Japan) were as follows: field sweep, 330.50–340.50 mT; field modulation frequency, 100 kHz; field modulation width, 0.1 mT; amplitude, 250; sweep time, 2 min; time constant, 0.1 s; microwave frequency, 9.420 GHz; and microwave power, 4 mW.

Superoxide Radical Scavenging Activity. Scavenging of superoxide anion from an HPX-XOD reaction system was assayed by the ESR measurement of the DMPO-OOH spin adduct (*24*). Fifty microliters of 2 mM HPX dissolved in 0.1 M PB, 50 μL of different concentrations of carotenoids dissolved in dimethylsulfoxide (DMSO) or of DMSO alone, 30 μL of DMSO, 20 μL of 4.45 M DMPO dissolved in pure water, and 50 μL of XOD dissolved to be 0.4 U/mL in 0.1 M PB were placed in a test tube and mixed. Each mixture was transferred to the ESR spectrometry cell, and the DMPO-OOH spin adduct was quantified 97 s after the addition of XOD. Signal intensities were evaluated from the peak height of the first signal of the DMPO-OOH spin adduct. Measurement conditions of ESR were as follows: field sweep, 330.50–340.50 mT; field modulation frequency, 100 kHz; field modulation width, 0.07 mT; amplitude, 200; sweep time, 2 min; time constant, 0.1 s; microwave frequency, 9.420 GHz; and microwave power, 4 mW. In an experiment for kinetic analyses with fucoxanthin by double reciprocal plots, different concentrations of DMPO were added to the system. Instead of different concentrations of fucoxanthin, different concentrations of SOD as a superoxide scavenger or of allopurinol as an XOD inhibitor were added to the system. The spin concentrations of DMPO-OOH were determined by the method described in previous papers (*23*, *25*). In brief, the double integrals of DMPO-OOH spectra (the area of the DMPO-OOH spectra) were compared with those of a TEMPOL standard (20 μM) measured under identical settings to estimate the concentrations of the DMPO-OOH adduct.

Singlet Oxygen Quenching Activity. Singlet oxygen was generated by a peroxidase, hydrogen peroxide, and halide system, (*26*) and the oxidation of DPIBF by the singlet oxygen was measured at 420 nm. The reaction mixture in acetate buffer (pH 4.5, 50 mM) contained 1 unit of peroxidase, 0.25 mM potassium bromide, 0.25 mM H_2O_2, 0.1 mM DPBIF solubilized in 0.05% Triton X-100, and the carotenoids in a suitable solvent. The reaction was initiated by addition of H_2O_2 into the thermostatically maintained cuvette containing the reaction mixture at 37 °C, and the decrease in absorbance at 420 nm was noted at 30 s intervals for 5 min. The quenching percent was calculated from the reduction in absorbance in the presence and absence of carotenoids. The quenching rate constant (k_Q) was determined by the method of Young et al. (*27*). Absorbance of the DPBIF against oxidation time was plotted for the control and for each concentration of the carotenoid, and the slope of the control (So) and carotenoid (Ss) was determined. So/Ss values were plotted against concentration of the carotenoid, and the slope of the regression line for each carotenoid (Sc) was determined. The rate constant (k_Q) was determined by the equation

$$\text{Sc} = 1 + (k_Q/k_d)$$

where k_d is the rate constant (2.5×10^5 s^{-1}) for natural decay of $^1O_2$ to $^3O_2$ in aqueous media (*28*).

Statistical Analysis. The difference between carotenoids in their ability to scavenge radicals and quench singlet oxygen was assessed statistically by ANOVA and Duncan's multiple range test using the STATISTICA software (*29*).

RESULTS AND DISCUSSION

Scavenging of DPPH, a stable free radical, is one of the major methods commonly used to evaluate the antioxidative activity. The method is based on the reduction of alcoholic DPPH in the presence of a hydrogen-donating antioxidant because of the formation of the nonradical form DPPH-H by the reaction (*30*). The DPPH scavenging activity of the three carotenoids tested is presented in **Figure 2**. The DPPH radical scavenging activity of the three carotenoids was found to be linearly dose dependent,

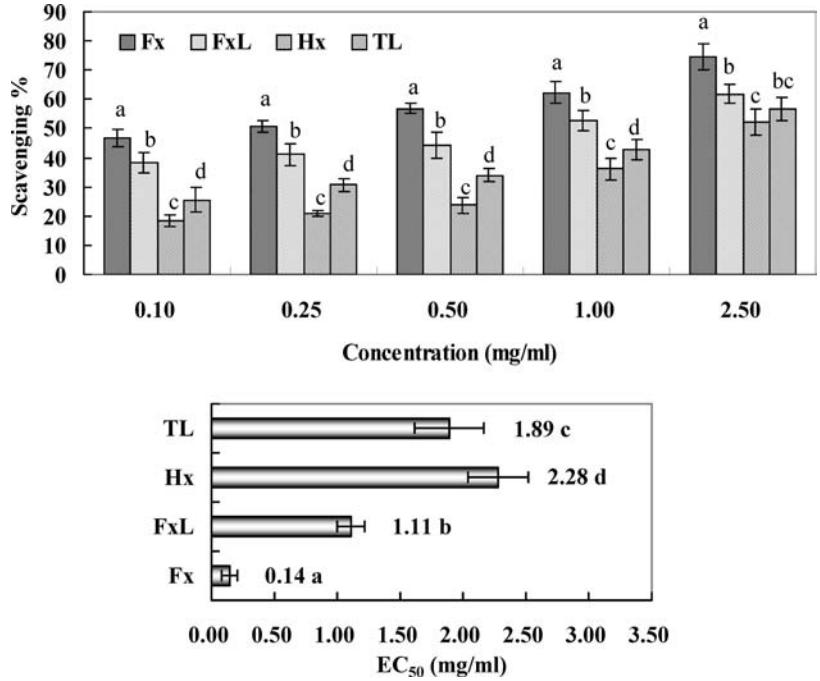

Figure 4. Hydroxyl radical scavenging activity of carotenoids and α-tocopherol as measured by the chemiluminescence technique. Fucoxanthin (Fx); fucoxanthinol (FxL); halocynthiaxanthin (Hx); α-tocopherol (TL; mean ± SD; $n = 4$). Bars with different superscripts for each concentration differ significantly ($p < 0.05$).

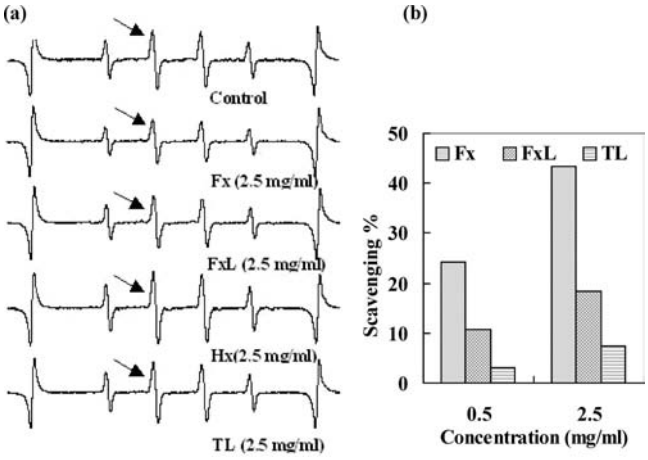

Figure 5. Typical ESR spectra of DMPO-OH• spin adduct and hydroxyl radical scavenging activity by fucoxanthin (Fx), fucoxanthinol (FxL), and α-tocopherol (TL).

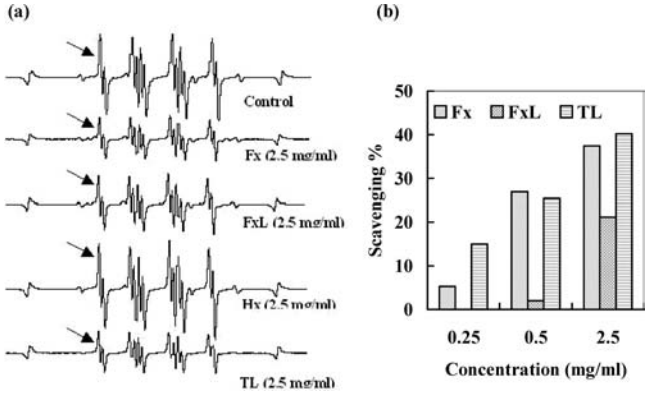

Figure 6. Typical ESR spectra of DMPO-OOH spin adduct and superoxide radical scavenging activity by fucoxanthin (Fx), fucoxanthinol (FxL), and α-tocopherol (TL).

with halocynthiaxanthin showing lower scavenging activity compared to fucoxanthin and fucoxanthinol. The scavenging activity was significantly different ($p < 0.05$) among the three carotenoids and the different concentrations of each carotenoid. However, when compared with that of α-tocopherol, the scavenging activity of these two carotenoids was lower. As the scavenging activity was linearly dependent on the concentration, the effective concentration for 50% scavenging (EC_{50}) was determined by using a linear regression equation. The EC_{50} of halocynthiaxanthin was significantly ($p < 0.05$) different, while for fucoxanthin and fucoxanthinol it was similar ($p > 0.05$). The EC_{50} values indicate that the scavenging activity of fucoxanthin and fucoxanthinol was 2.6 and 2.4 times lower, respectively, while that of halocynthiaxanthin was 12.9 times lower than that of α-tocopherol. Nishino (*19*) had observed by the ESR technique a 28% scavenging of DPPH radical by 0.5 mM fucoxanthin. Fucoxanthin was found to be a more potent DPPH radical scavenger than other carotenoids such as β-carotene, β-cryptoxanthin, zeaxanthin, and lutein under anoxic conditions than under aerobic conditions, but it always had a lower activity than α-tocopherol (*18*).

The ABTS radical scavenging assay is one of the popular indirect methods of determining the antioxidative capacity of compounds (*31*). In the absence of antioxidants, the ABTS radical is rather stable, but it reacts energetically with an H atom donor and is converted into a noncolored form of ABTS. The ABTS radical scavenging activity of fucoxanthinol was found to be higher than that of the other two carotenoids and even

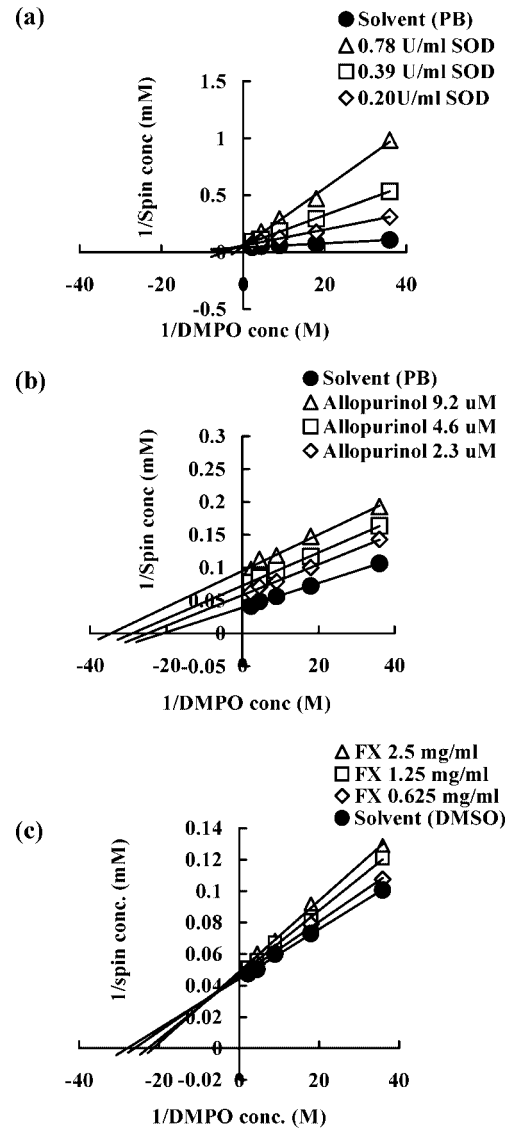

Figure 7. Double reciprocal plots of formation of DMPO-OOH vs DMPO concentrations at different fixed concentration of (**a**) SOD, (**b**) allopurinol, and (**c**) fucoxanthin.

higher than that of α-tocopherol (**Figure 3**). However, the difference in EC_{50} values among fucoxanthin, fucoxanthinol, and α-tocopherol was marginal ($p > 0.05$). The EC_{50} values calculated from the regression equations show that the order of potency of the samples tested for ABTS radical scavenging was fucoxanthinol > α-tocopherol > fucoxanthin > halocynthiaxanthin. It has been reported that the presence of functional groups such as carbonyl and hydroxyl groups in the terminal rings reduces the ABTS scavenging activity of carotenoids (*32*). Even though fucoxanthinol has three hydroxyl groups compared to two groups in fucoxanthin and halocynthiaxanthin, it exhibited higher activity. The presence of an allenic bond may be responsible for the higher activity of fucoxanthinol and fucoxanthin compared to that of halocynthiaxanthin. Further, an additional acetyl group in fucoxanthin may be responsible for the reduction of its ABTS scavenging activity compared to that of fucoxanthinol.

The hydroxyl radical (•OH) was generated by the Fenton reaction system, and the scavenging of these radicals by carotenoids was evaluated by chemiluminescence and ESR techniques. The hydroxyl radical scavenging activity as measured by the chemiluminescence technique (**Figure 4**) indicates

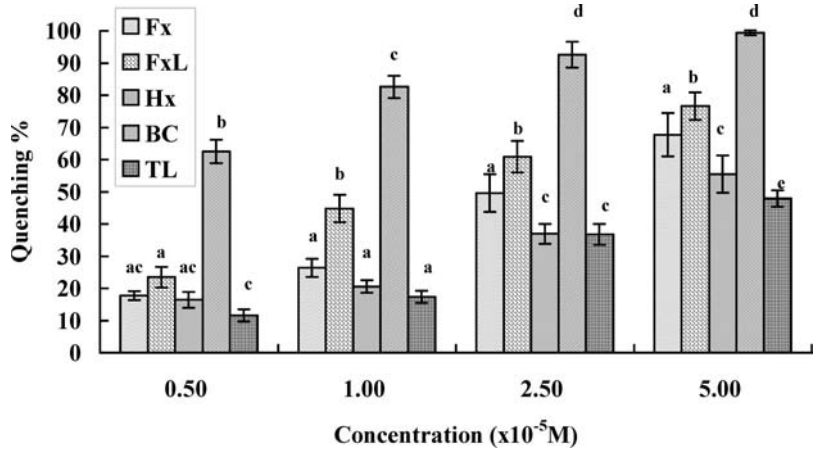

Figure 8. Singlet oxygen quenching activity of carotenoids and α-tocopherol. Fucoxanthin (Fx); fucoxanthinol (FxL); halocynthiaxanthin (Hx); β-carotene (BC); α-tocopherol (TL; mean ± SD; $n = 4$). Bars with different superscripts for each concentration differ significantly ($p < 0.05$).

that fucoxanthin has the strongest activity followed by fucoxanthinol. Even at a 0.10 mg/mL concentration, fucoxanthin showed a 46.72 ± 3.11% scavenging activity compared to 38.33 ± 3.67% by fucoxanthinol, 18.57 ± 1.92% by halocynthiaxanthin, and 25.49 ± 4.30% by α-tocopherol at the same concentration. DMSO, the known hydroxyl radical scavenger, always showed more than 95% scavenging activity. The scavenging activity among the three carotenoids differed significantly ($p < 0.05$) at all concentrations tested. The EC_{50} values calculated from the linear regression equation for scavenging percent versus concentration indicate that scavenging activity by fucoxanthin was 7.9 times higher than for fucoxanthinol, 16.3 times higher than for halocynthiaxanthin, and 13.5 times higher than for α-tocopherol.

In the ESR technique for analysis of hydroxyl radical scavenging, the hydroxyl radical generated by the Fenton system was trapped by the spin-trapping agent DMPO, and the signal intensity of the resultant DMPO-OH$^{\bullet}$ spin adduct was measured (**Figure 5a**). To assess the activity of carotenoids, the signal intensity of the DMPO-OH spin adducts was measured in the presence and absence of carotenoids at two different levels. The reduction in the signal intensity of the spin-trapped radical in the presence of the test compound indicates the scavenging activity. The trend for radical scavenging was similar to that observed with the chemiluminescence technique. The signal intensity of DMPO-OH$^{\bullet}$ spin adducts was lower in the presence of fucoxanthin (56.6% of control) compared with fucoxanthinol (81.7%) and α-tocopherol (92.5%) at a concentration of 2.5 mg/mL. This indicates a higher reduction in signal intensity in presence of fucoxanthin and, thus, a higher scavenging activity. The scavenging activity of fucoxanthin, fucoxanthinol, and α-tocopherol, each at 2.5 mg/mL, corresponds to 43.4, 18.3, and 7.5%, respectively (**Figure 5b**). Halocynthiaxanthin did not show any reduction in signal intensity, indicating no scavenging activity. The percent scavenging observed was lower with the ESR technique compared with the luminescence technique. A similar difference between the two techniques has been observed while analyzing the hydroxyl radical scavenging activity of zeaxanthin and lutein by these two methods (*33*). It has been pointed out that the chemical systems used may produce a complex of different reactive oxygen species that are all based on $^{\bullet}$OH production, which interferes with the luminescence measurements (*33*).

The ability of carotenoids to scavenge the superoxide anion was measured with the ESR technique. The superoxide anion was generated enzymatically with the hypoxanthin–xanthine oxidase (HPX-XOD) system, and the generated radical was trapped by DMPO; the signal intensity of the resultant DMPO-OOH$^{\bullet}$ (**Figure 6a**) was measured in the presence and absence of the carotenoid. As an $^{\bullet}$OH radical is also produced in the HPX-XOD system, DMSO was used as a scavenger of the $^{\bullet}$OH radical (*34, 35*). Halocynthiaxanthin did not exhibit any reduction in signal intensity, while the signal intensity in the presence of 2.5 mg/mL fucoxanthin (62.6% of control) was lower than that of fucoxanthinol (74.8%) and was almost similar to that of α-tocopherol (59.8%), indicating higher superoxide scavenging activity by fucoxanthin. The reduction in signal intensity corresponds to 37.4, 21.2, and 40.2% scavenging by fucoxanthin, fucoxanthinol, and α-tocopherol, respectively, each at a concentration of 2.5 mg/mL (**Figure 6b**).

The signal intensity of DMPO-OOH$^{\bullet}$ also reduces when a compound inhibits the activity of the enzyme in the HPX-XOD system (*36*). To test the reduction in the signal intensity of DMPO-OOH$^{\bullet}$ caused by scavenging or inhibition, the use of a competitive reaction between the trapping agent or the inhibitor and the active oxygen is suggested (*23, 37*). As fucoxanthin showed a higher reduction in signal intensity of DMPO-OOH and to confirm whether the reduction is due to scavenging of the radical or interference with the enzyme reaction of HPX-XOD, the competitive reaction between DMPO and fucoxanthin or reference agents was evaluated as per the protocol reported earlier (*23*). The reference agents used were SOD as a superoxide scavenger and the drug allopurinol as an enzyme inhibitor. The double reciprocal plot for SOD, allopurinol, and fucoxanthin is depicted in **Figure 7**. The linear and intersecting patterns of the double reciprocal plots indicate that SOD acted as a competitive inhibitor of DMPO (**Figure 7a**). On the other hand, the double reciprocal plots show that the inhibition of the DMPO-OOH formation by allopurinol was uncompetitive with DMPO (**Figure 7b**). As for fucoxanthin (**Figure 7c**), the reduction in signal intensity of DMPO-OOH in the presence of fucoxanthin is mainly due to scavenging of the radical directly. However, as the intersection is shifted marginally to the negative side of the *x* axis, it can also be assumed that the carotenoid also interferes with the enzyme system.

The singlet oxygen quenching activity of the three carotenoids was compared with that of β-carotene and α-tocopherol (**Figure 8**). β-Carotene showed significantly ($p < 0.05$) high quenching activity (62.5%) even at a low concentration of 0.5 μM. Fucoxanthinol showed relatively higher quenching activity than fucoxanthin and halocynthiaxanthin. The quenching rate constant (k_Q) was determined from the slope of the plot of So/Ss against

Table 1. Singlet Oxygen Quenching Rate Constant (k_Q) of Carotenoids[a]

carotenoid	double bonds	functional groups	k_Q ($\times 10^{10}$ M^{-1} s^{-1})	relative k_Q[b]
fucoxanthin	10 (1 allenic bond)	2 OH; 1 epoxy; 1 carbonyl; 1 acetyl	1.19 ab	2.4
fucoxanthinol	10 (1 allenic bond)	3 OH; 1 epoxy; 1 carbonyl	1.81 a	3.7
halocynthiaxanthin	10 (1 triple bond)	2 OH; 1 epoxy; 1 carbonyl	0.80 ab	1.6
β-carotene	11		12.78 c	26.2
α-tocopherol			0.49 b	1.0

[a] k_Q values with different letters differ significantly ($p < 0.05$). [b] Relative to α-tocopherol.

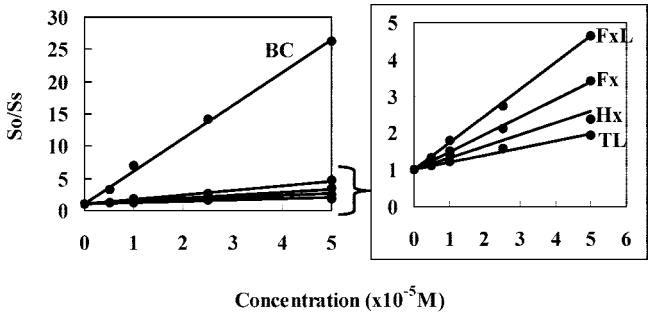

Figure 9. Plot of So/Ss vs concentration of fucoxanthin (Fx), fucoxanthinol (FxL), halocynthiaxanthin (Hx), β-carotene (BC), and α-tocopherol (TL).

concentration (**Figure 9**). The rate constant for β-carotene was comparatively much higher than for fucoxanthin and its metabolites (**Table 1**). However, the difference in quenching rate among fucoxanthin and its two metabolites was marginal ($p < 0.05$). The singlet oxygen quenching rate of β-carotene was faster by 10.7 times that of fucoxanthin, 7.1 times that of fucoxanthinol, 16.0 times that of halocynthiaxanthin, and 26.2 times that of α-tocopherol.

The singlet quenching activity of a carotenoid is influenced by the number of conjugated double bonds (*38*). The reported values for the carotenoids vary as the experimental conditions varied. Astaxanthin and spirilloxanthin, with 13 double bonds, were found to exhibit a higher singlet oxygen quenching activity than β-carotene (*6*). However, the same work did not observe much difference between fucoxanthin and β-carotene in their ability to quench singlet oxygen, and it concluded that the presence of conjugated keto groups increases the quenching rate while the presence of a hydroxyl, epoxy, and methoxy group has lesser effects. Even though fucoxanthin and its two metabolites have a conjugated keto group, their effects were not comparable to that of β-carotene. The presence of hydroxyl and epoxy groups and a lesser number of double bonds may be responsible for the lower quenching effects of these carotenoids. Among the three carotenoids, fucoxanthin and fucoxanthinol, both having an allenic bond, showed higher quenching activity than halocynthiaxanthin.

The antioxidant activity of carotenoids is related to their structure (*39*). The number of double bonds and the presence of functional groups influence the interaction of carotenoids with different radicals (*40*). It is also observed that the carotenoid–radical reactions not only depend on the carotenoids but also on the nature of the radical (*41*). Depending on the nature of the radical, the mechanism of scavenging may be by electron donation, resulting in the formation of a carotenoid radical cation, and/or by generation of a carotenoid–radical adduct (*41*). It was also reported that degradation products of different carotenoids have different pro- and antioxidant effects (*42*). Among the three carotenoids tested, fucoxanthin and fucoxanthinol showed a higher radical scavenging and singlet quenching activity than halocynthiaxanthin. The scavenging activity of these two carotenoids was also higher or similar to that of α-tocopherol. The order of scavenging activity of each carotenoid towards a different radical followed a similar pattern of fucoxanthin > fucoxanthinol > halocynthiaxanthin, except for the ABTS radical, where fucoxanthinol showed higher activity than fucoxanthin. However, the radical scavenging and singlet oxygen quenching activity between fucoxanthin and fucoxanthinol did not show any significant difference. The major structural differences in these three carotenoids are the presence of an allenic bond in fucoxanthin and fucoxanthinol and of an acetyl group in the terminal ring of fucoxanthin. Hence, it may be assumed that the allenic bond is responsible for the higher antioxidant activity of fucoxanthin and fucoxanthinol. Further studies on the mechanism and rate of radical scavenging by these carotenoids would provide the role of these structural features in the antioxidant activity. Even though halocynthiaxanthin showed very poor scavenging activity, it has been reported that halocynthiaxanthin shows the highest suppressive effect on the generation of free radicals in stimulated leucocytes (*43*). The reaction of carotenoids with radicals also depends on factors such as oxygen pressure particularly in biological systems, resulting in a shift towards pro-oxidant behavior (*44*). Thus, it is important that in vitro antioxidant activity of carotenoids also be confirmed with in vivo experiments.

It can be summarized from this study that the seaweed carotenoids, fucoxanthin and its metabolite fucoxanthinol, exhibit antioxidant activities comparable to that of α-tocopherol. Halocynthiaxanthin, another metabolite of fucoxanthin, showed comparatively lower antioxidant activities. It is presumed that the presence of an allenic bond in fucoxanthin and fucoxanthinol and of an acetyl functional group in fucoxanthin is responsible for the higher antioxidant activities.

LITERATURE CITED

(1) Darley-Usmar, V.; Halliwell, B. Blood radicals: relative nitrogen species, relative oxygen species, transition metal ions and the vascular system. *Pharm. Res.* **1996**, *13*, 649–662.
(2) Halliwell, B. Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. *Free Radical Res.* **1996**, *25*, 57–74.
(3) Elliot, R. Mechanisms of genomic and non-genomic actions of carotenoids. *Biochim. Biophys. Acta* **2005**, *1740*, 147–154.
(4) Kiokias, S.; Gordon, M. H. Antioxidant properties of carotenoids in vitro and in vivo. *Food Rev. Int.* **2004**, *20*, 99–121.
(5) Palozza, P.; Krinsky, N. I. Antioxidant effects of carotenoids in vivo and in vitro: an overview. *Methods Enzymol.* **1992**, *213*, 403–420.
(6) Hirayama, O.; Nakamura, K.; Hamada, S.; Kobayashi, Y. Singlet oxygen quenching ability of naturally occurring carotenoids. *Lipids* **1994**, *29*, 149–150.
(7) Partali, V.; Tangen, K.; Liaaen-Jensen, S. Carotenoids in food chain studies: III. Resorption and metabolic transformation of carotenoids in Mytilus edulis (edible mussel). *Comp. Biochem. Physiol.* **1989**, *92B*, 239–246.
(8) Ookubo, M.; Matsuno, T. Carotenoids of sea squirts II. Comparative biochemical studies of carotenoids in sea squirts. *Comp. Biochem. Physiol.* **1985**, *81B*, 137–141.

(9) Sugawara, T.; Baskaran, V.; Tsuzuki, W.; Nagao, A. Brown algae fucoxanthin is hydrolyzed to fucoxanthinol during absorption by Caco-2 human intestinal cells and mice. *J. Nutr.* **2002**, *132*, 946–951.

(10) Kotake-Nara, E.; Kushiro, M.; Zhang, H.; Sugawara, T.; Miyashita, K.; Nagao, A. Carotenoids affect proliferation of human prostrate cancer cells. *J. Nutr.* **2001**, *131*, 3303–3306.

(11) Hosokawa, M.; Wanezaki, S.; Miyauchi, K.; Kurihara, H.; Kohno, H.; Kawabata, J.; Odashima, S.; Takahashi, K. Apoptosis-inducing effect of fucoxanthin on human leukemia cell HL-60. *Food Sci. Technol. Res.* **1999**, *5*, 243–246.

(12) Hosokawa, M.; Kudo, M.; Maeda, H.; Kohno, H.; Tanaka, T.; Miyashita, K. Fucoxanthin induces apoptosis and enhances the antiproliferative effect of PPARγ ligand, troglitazone, on colon cancer cells. *Biochim. Biophys. Acta* **2004**, *1675*, 113–119.

(13) Ikeda, K.; Kitamura, A.; Machida, H.; Watanabe, M.; Negishi, H.; Horaoka, J.; Nakano, T. Effect of Undaria pinnatifida (Wakame) on the development of cardiovascular disease in stroke-prone spontaneously hypertensive rats. *Clin. Exp. Pharmacol. Physiol.* **2003**, *30*, 44–48.

(14) Shiratori, K.; Ohgami, K.; Ilieva, I.; Jin, X. H.; Koyama, Y.; Miyashita, K.; Kase, S.; Ohno, S. Effects of fucoxanthin on lipopolysachharide-induced inflammation in vitro and in vivo. *Exp. Eye Res.* **2005**, *81*, 422–428.

(15) Maeda, H.; Hosokawa, M.; Sashima, T.; Funayama, K.; Miyashita, K. Fucoxanthin from edible seaweed Undaria pinnatifida, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochem. Biophys. Res. Commun.* **2005**, *332*, 392–397.

(16) Maeda, H.; Hosokawa, M.; Sashima, T.; Takahashi, N.; Kawada, T.; Miyashita, K. Fucoxanthin and its metabolite, fucoxanthinol, suppress adipocyte differentiation in 3T3-L1 cells. *Int. J. Mol. Med.* **2006**, *18*, 147–152.

(17) Konishi, I.; Hosokawa, M.; Sashima, T.; Kobayashi, H.; Miyashita, K. Halocynthiaxanthin and fucoxanthinol isolated from Halocynthia roretzi induce apoptosis in human leukemia, breast and colon cancer cells. *Comp. Biochem. Physiol.* **2005**, *142*, 53–59.

(18) Nomura, T.; Kikuchi, M.; Kubodera, A.; Kawakami, Y. Proton-donative antioxidant activity of fucoxanthin with 1,1-Diphenyl-2-Picrylhydrazyl (DPPH). *Biochem. Mol. Biol. Int.* **1997**, *42*, 361–370.

(19) Nishino, H. Cancer prevention by carotenoids. *Mutat. Res.* **1998**, *402*, 159–163.

(20) Yan, X.; Chuda, Y.; Suzuki, M.; Nagata, T. Fucoxanthin as the major antioxidant in Hijkia fusiformis, a common edible seaweed. *Biosci., Biotechnol., Biochem.* **1999**, *63*, 605–607.

(21) Duan, X. J.; Zhang, W. W.; Li, X. M.; Wang, B. G. Evaluation of antioxidant property of extract and fractions obtained from red alga Polysiphonia urceolata. *Food Chem.* **2006**, *95*, 37–43.

(22) Komrskova, D.; Lojek, A.; Hrbac, J.; Ciz, M. A comparison of chemical systems for luminometric determination of antioxidant capacity towards individual reactive oxygen species. *Luminescence* **2006**, *21*, 239–244.

(23) Niwano, Y.; Sato, E.; Kohno, M.; Matsuyama, Y.; Kim, D.; Oda, T. Antioxidant properties of aqueous extracts from red tide plankton cultures. *Biosci., Biotechnol., Biochem.* **2007**, *71*, 1145–1153.

(24) Sato, E.; Kohno, M.; Hamano, H.; Niwano, Y. Increased anti-oxidative potency of garlic by spontaneous short-term fermentation. *Plant Foods Hum. Nutr.* **2006**, *61*, 157–160.

(25) Kohno, M.; Mizuta, Y.; Kusai, M.; Masumizu, T.; Makino, K. Measurements of superoxide anion radical and superoxide scavenging activity by electron spin resonance spectroscopy coupled with DMPO spin trapping. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 1085–1090.

(26) Piatt, J. F.; Cheema, A. S.; O'Brien, P. J. Peroxidase catalysed singlet oxygen formation from hydrogen peroxide. *FEBS Lett.* **1977**, *74*, 251–254.

(27) Young, R. H.; Brewer, D.; Keller, R. A. The determination of rate constants of reaction and lifetimes of singlet oxygen in solution by a flash photolysis technique. *J. Am. Chem. Soc.* **1971**, *95*, 375–379.

(28) Lindig, B. A.; Rodgers, M. A. J. Laser photolysis studies of singlet molecular oxygen in aqueous micellar dispersions. *J. Phys. Chem.* **1979**, *83*, 1683–1688.

(29) Statsoft Inc. *STATISTICA for windows*; Statsoft Inc.: Tulsa, OK, **1999**.

(30) Shon, M. Y.; Kim, T. H.; Sung, N. J. Antioxidants and free radical scavenging activity of *Phellinus baumii* (*Phellinus* of Hymenochaetaceae) extracts. *Food Chem.* **2003**, *82*, 593–597.

(31) Roginsky, V.; Lissi, E. A. Review of methods to determine chain-breaking antioxidant activity in food. *Food Chem.* **2005**, *92*, 235–254.

(32) Miller, N. J.; Sampson, J.; Candeias, L. P.; Bramley, P. M.; Rice-Evans, C. A. Antioxidant activities of carotenes and xanthophylls. *FEBS Lett.* **1996**, *384*, 240–242.

(33) Trevithick-Sutton, C. C.; Foote, C. S.; Collins, M.; Trevithick, J. R. The retinal carotenoids zeaxanthin and lutein scavenge superoxide and hydroxyl radicals: A chemiluminescence and ESR study. *Mol. Vision* **2006**, *12*, 1127–1135.

(34) Hodgson, E. K.; Fridobvich, I. The role of O_2^- in the chemiluminescence of luminol. *Photochem. Photobiol.* **1973**, *18*, 451–455.

(35) Sato, M.; Niwano, Y.; Mokudai, T.; Kohno, M.; Matsuyama, Y.; Kim, D.; Oda, T. A discrepancy in superoxide scavenging activity between the ESR spin trapping method and the luminol chemiluminescence method. *Biosci., Biotechnol., Biochem.* **2007**, *71*, 1505–1513.

(36) Kimura, K. Mechanisms of active oxygen species reduction by non-steroidal anti-inflammatory drugs. *Int. J. Biochem. Cell Biol.* **1997**, *29*, 437–446.

(37) Mitsuta, K.; Mizuta, Y.; Kohno, M.; Hiramatsu, M.; Mori, A. The application of ESR spin-trapping technique to the evaluation of SOD-like activity of biological substances. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 187–191.

(38) Di Mascio, P.; Murhy, M. E.; Sies, H. Antioxidant defence systems: the role of carotenoid, tocopherol and thiols. *Am. J. Clin. Nutr.* **1991**, *53*, 194S–200S.

(39) Mordi, R. C. Carotenoids-function and degradation. *Chem. Ind.* **1993**, *110*, 79–83.

(40) Woodall, A. A.; Lee, S. W.; Weesie, R. J.; Jackson, M. J.; Britton, G. Oxidation of carotenoids by free radicals: relationship between structure and reactivity. *Biochim. Biophys. Acta* **1997**, *1336*, 33–42.

(41) Mortensen, A.; Skibsted, L. H.; Sampson, J.; Rice-Evans, C.; Everett, S. A. Comparative mechanisms and rates of free radical scavenging by carotenoid antioxidants. *FEBS Lett.* **1997**, *418*, 91–97.

(42) Jorgensen, K.; Skibsted, L. H. Carotenoids scavenging of radicals. *Z. Lebensm. Unters. Forsch. A* **1993**, *196*, 423–429.

(43) Murakami, A.; Nakashima, M.; Koshiba, T.; Maoka, T.; Nishino, H.; Yano, M.; Sumida, T.; Kim, O. K.; Koshimizu, K.; Ohigashi, H. Modifying effects of carotenoids on superoxide and nitric oxide generation from stimulated leucocytes. *Cancer Lett.* **2000**, *149*, 115–123.

(44) Haila, K. M.; Nielsen, B. R.; Heinon, M. I.; Skibsted, L. H. Carotenoid reaction with free radicals in acetone and toluene at different oxygen partial pressures. *Z. Lebensm.-Unters. Forsch A.* **1997**, *204*, 81–87.

Received for review June 22, 2007. Revised manuscript received August 6, 2007. Accepted August 6, 2007. N.M.S. is grateful to the Japanese Society for Promotion of Science (JSPS) for the award of a postdoctoral fellowship. This work was supported by the Research and Development Program, Bio-oriented Technology Research Advancement Institution, Government of Japan.

JF071848A